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ASSESSMENT OF ACUTE AND SUB-ACUTE TOXICITY OF OLIVE POMACE IN FEMALE WISTAR RATS

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Abstract – *Objective:* *Olive Pomace (OP) is considered to be a rich source of phenolic compounds. Recently many researches showed a broad biological activity of this by-product of the olive oil production process in addition to their emergence as value-added materials with potential applications in the pharmaceutical, food, and nutraceutical industries. The present study is aimed to evaluate* in vivo *toxicological activities of OP.*

Materials and Methods: *The qualitative phytochemical analysis aims to determine the key phytoconstituents found in OP. For the* in vivo *study, two types of tests are performed: acute and 28-day repeated oral toxicity studies in Wistar rats for evaluation of hematological, biochemical, and histological parameters.*

Results: *The qualitative phytochemical analysis has revealed the presence of polyphenols, flavonoids, tannins, saponins, quinones, anthraquinones, terpenoids, and compounds reduced in our methanol extract of OP. In acute oral toxicity, no treatment-related death or toxic signs are observed in female rats for 14 days in 200, 2000, 3000, and 5000 mg/kg doses, besides LD50 value is found to be up to 2000 mg/kg bodyweight. As for the Globally Harmonized System of Classification and Labelling of Chemicals. 28-days sub-acute toxicity study is carried in female rats at four dose levels (3.12, 31.25, 125 and 500 mg/kg), no changes in observation related death and toxic signs when compared with control. The hematological and biochemical investigation shows a significant change* ($p>0.05$) *in the high-level doses (500 mg/kg).*

Conclusions: *According to the findings of this study, OP extract has the potential to be used to generate new anti-cancer and antioxidant additives for pharmaceutical and food manufacturing. Long-term in vivo toxicological tests should also be conducted to determine a safe dosage of OP extract.*

KEYWORDS: *Olive pomace, Methanol extract, Acute toxicity, Sub-acute toxicity.*

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INTRODUCTION

Complementary and alternative medicine (CAM) is defined as the use of practices and biological substances in place and together with conventional medicine, including cancer patients[1-3]. With the rapid development of the olive fruit processing industry around the world, Olive Pomace (OP) as a waste from the olive oil industry is respectively increasing, with massive quantities produced each year[4]. However, only small amounts of olive mill waste are re-used, mainly as fertilizers, biomass, or additive in animal food, while a large quantity remains without actual application[5].

Recent research has demonstrated that OP has broad biological activities, such as anti-inflammatory, hepatoprotective, gastro protective, anti-ulcer, anti-HIV, anticancer, anti-diabetic, hypolipidaemic, antiatherosclerotic and immunoregulatory effects [6]. A wide range of phenolic compounds have been identified in virgin oil, including phenolic alcoholic, secoirdoid derivatives, phenolic acids and flavonoids. However, only about 2% of the total phenols found in olive fruits are transferred to the extracted olive oil, while the other 98% are retained in the olive cake. The analysis of these phenolic extracts has demonstrated their high antioxidant and anticancer activity and suggested their potential use as additives for the food industry[7].

Olive cake has been considered to be an interesting biological source of phenolic compounds[8], without Bisphenol A (additive for polycarbonate plastics), the main cause of endocrine disruption associated to cancer risk and cardiovascular diseases[9,10]. Several researches focus on the extraction optimization of these secondary metabolites activities, proved by different *in vivo* pharmacological studies[11].

The use of small laboratory animals such as the rat is convenient and very essential to perform toxicological studies[12,13] in order to identify adverse effects and to determine limits of exposure level at which such effects occur[14]. Some doses have beneficial effects but increasing exposure will, at some point, cause harmful effect and the substance is considered toxic at that level [15]. Toxicological research and testing help to live safely and predict benefit from synthetic and natural substance, avoiding harm[13].

To validate the use of this phenolic extracts and to demonstrate their impact in the healthcare, toxicological studies are necessary. Conventionally in the presence of an unknown substance, the first step in the search for pharmacological activity begins with the study of toxicity and in particular by evaluation of the lethal dose 50 (DL 50)[16].

The border between drug and toxic is vague. It is often a question of dose[17]. Despite the widespread use of OP in pharmaceutical, food and nutraceutical industries and to ensure their safety, it's necessary to determine the application dose through the sub chronic toxicity study. There is a lack of experiment al data on possible toxicity of OP. To avoid this situation, the present study aims to search for OP safety data focusing on acute toxicity and 28-day sub chronic toxicity of hydro methanolic extract of OP administered orally in female Wistar rat. The present study aims to test *in vivo* toxicological activities of hydromethanolic OP extract.

MATERIALS AND METHODS

Plant material

OP WAS collected from Tlemcen in Northwest of Algeria; the plant has been identified in the Laboratory (PPABIONUT) of the Department of Biology, University of Tlemcen.

Preparation of extract

OP was dried in ambient air in the room, sheltered from the sun then finely powdered. 50 g of OP powder were macerated in 800 ml of methanol and 200 ml of distilled water for 24 h on a shaker at 100 rpm, the resultant extract filtered through a fine muslin cloth, and then a filter paper Whatman (Grade 1-Circles, 150 mm). The filtrate was dried by using Eyela rotary evaporator at 45°C until getting powder. This methanol extract dissolved in 5% of DMSO before use.

Phytochemical study

The qualitative phytochemical study was performed to identify the main phytoconstituents[18] present in the OP by using standard procedures as described by Trease and Evans[19] and Harborne et al[20].

Animals

Females Albino Wistar rats where used for the acute and sub-acute oral toxicology tests, obtained from Pasteur Laboratories (Alger, Algeria). The rats were adult (8-10 weeks) weigh between 140 g-200 g. They were raised at ambient temperature of 22±3°C with 40 to 50% moisture and a photoperiod of 12 hours light and 12 hours darkness[18]. Experimental protocols adopted were based on World Health Organization Guidelines for care and use of laboratory animals. The experimental usage of the

animals was approved by the Ethics Committee of the National Ethics Committee for Control and Supervision of Experiments on Animals. The animals were distributed into five groups of 3 rats each for acute oral toxicity and four groups of 5 rats each for sub-acute oral toxicity.

Acute oral toxicity

Healthy female Wistar rats were used in this study according to the instructions of the Organization for Economic Cooperation and Development (OECD) for acute oral toxicity tests[21]. All animals were fasted overnight, but with free access to water and weighed before administration of the extract. 15 females SD rats were randomly divided into 5 groups of 3 rats each, group1 (negative control) was administrated DMSO (5%) and saline solution. Four groups test was administrated methanol OP extract at dose 200, 2000, 3000 and 5000 mg/kg body weight of OP extract respectively. The observations focused on mortality, convulsions, salivations, tremors, sleep and coma each day for 14 days. The body weights of animals recorded shortly before the administration of the tested substance and at the end of each week.

Sub acute oral toxicity

For this study, the rats (30 females) were divided into five groups of five each; four of these lots were treated with the different doses of the extract (3.12, 31.25, 125 and 500 mg/kg) and the fifth was administered with the vehicle (control) according to the OECD 407 guideline[22]. The OP extract was administered by gavage (1 ml/100 g) daily for 28 consecutive days. Mortality, food consumption and water, body weight and observation for general toxicity signs of the animals were recorded daily. At the end of the experiment, the animals were sacrificed to collect their blood for biochemical and hematological analyzes. Organs such as liver, kidneys and white adipose tissue were collected, washed immediately in NaCl (0.9%), weighed individually and examined macroscopically. The organs removed were weighed and the relative weight of the organs was calculated. The percentage of body weight change of the animals' model calculated according to the following equation:

$$\frac{\text{Body weight at the end of each week-initial body weight}\times 100}{\text{Initial body weight}}$$

After the blood collection, the vital organs (liver, kidney, spleen and pancreas were cleaned of fat and blotted with clean tissue paper, and then weighed on balance. The relative organ's weights (ROWs) were calculated and rec recorded in proportion to the body weight according to the following equation:

$$\text{ROW} = \frac{\text{Absolute organ weight}}{\text{Body weight at sacrifice}} \times 100$$

Blood analysis

Biochemical analyzes included glycemia, total cholesterol, triglycerides, HDL, liver function markers (AST, ALT), and nephrotic markers (urea, creatinine), using Spinreact Kit. Hematologic parameters included red and white blood cells, hemoglobin, hematocrit, MPV, MCH, MCHC, platelets, lymphocytes, monocytes, eosinophils, neutrophils and basophils, with the unit XN-1000 (Sysmex) [18], according to the manufacturer's instructions (Wakinohama-Kaigandori, Chuo-Ku, Kobe, Hyogo, Japan).

Histological examination

Tissue samples from kidney, liver and white adipose tissue were collected and fixed in 10% phosphate-buffered formalin (pH 7.0) for 24 h, routinely processed, embedded in paraffin wax, cut into 2–3 µm (µm) sections and stained with hematoxylin and eosin. All the stained sections were examined for optical microscopy using Olympus microscope (Tokyo, Japan).

Statistical analysis

Statistical results are represented as mean±standard error of mean (SEM). The differences between groups of acute and sub-acute toxicity tests determined by one way analysis of variance ANOVA (Minitab 18). The value $p<0.05$ was considered significant.

RESULTS

Phytochemical study

Results of phytochemical screening are presented in Table 1. The phytochemical screening of methanol extract of OP showed the presence of bioactive phytocompounds such as phenols, flavonoids, tannins, saponins, quinones, terpenoids and compounds reducing.

TABLE 1. Phytochemical screening of methanol extract of *Pomace Olive*.

Phytochimical compounds	*Test used*	*Extract of Pomace Olive*
Polyphénols	Ferric chloride FeCl3 (2%)	+++
Flavonoids	hydrochloric acid +magnésium	++
Tannins	Ferric chloride FeCl3 (1%)	++
Saponins	Foam test	+++
Quinones	Sodium hydroxide (1%)	++
Anthraquinones	ammonium hydroxide NH4OH (10 %)	–
Terpenoids	Chloroform+Sulfiric acid	++
Compounds reducing	Fehling liqueur	+

(-): Absence ;(+): Trace ;(++): Low presence ;(+++): high presence

Acute toxicity test

The acute toxicity results showed no evidence of toxicity of the methanolic extract of OP administered at the 200 mg/kg, 2000 mg/kg, 3000 mg/kg and 5000 mg/kg dose limit in animals during the observation period (14 days). The rats survived until the end of the observation period. Physical observation of the groups treated rats for acute oral toxicity tests throughout this study indicated that there is no sign of toxic effects in all groups.

No abnormalities were found in the organs at autopsy, hematological and biochemical parameters. The body weight did change significantly (p<0.05) during the 14 days period between the PO treated groups rats had and the control. In addition, the percentage changes in body weight of the PO treated groups were significantly different compared to the control rats as (p<0.05) (Figure1). The results on the relative weight of organs showed non-significant difference (p> 0.05) between the different doses administered and the control on the liver, the kidney and the spleen. However, there was significantly different (p<0.05) on the pancreas (Figure 2).

Sub-acute toxicity test

Administration of various doses of methanol extract of OP (3.12, 31.25, 125 and 500 mg/kg) during 28 days of treatment had significant change (p<0.05) on the body weight of treated rats compared to controls in sub-acute oral toxicity (Figure 3). The results on the relative organs weight showed non-significant difference (p>0.05) in groups treated compared to control on the pancreas and the kidney. On liver, the analyses reveal a significant difference (p<0.05) and on the spleen (p>0.05) in all treatment doses compared to control (Figure 4). For the biochemical parameters of oral sub-acute toxicity recorded in Table 2. The results showed no significant difference in ALT and total protein (p<0.05) between all treated groups and control. On the other hand, the parameters such as glucose, triglycerides, total cholesterol, HDL, AST, urea and creatinine recorded significant differences (p<0.05), depending on the dose administered. Statistical analyses of hematological parameters showed no significant difference on several parameters (hematocrit, hemoglobin, platelets, MPV, MCH, neutrophils, monocytes, lymphocytes and basophils.) in both control and treated groups.

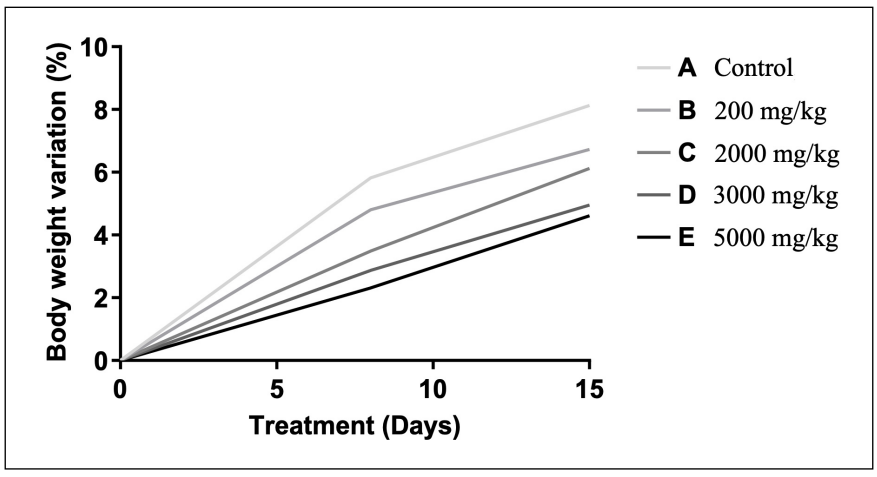

Fig. 1. Effect on the treatment with the methanol extract of olive pomace on the variation of body weight of rats in acute oral toxicity test.

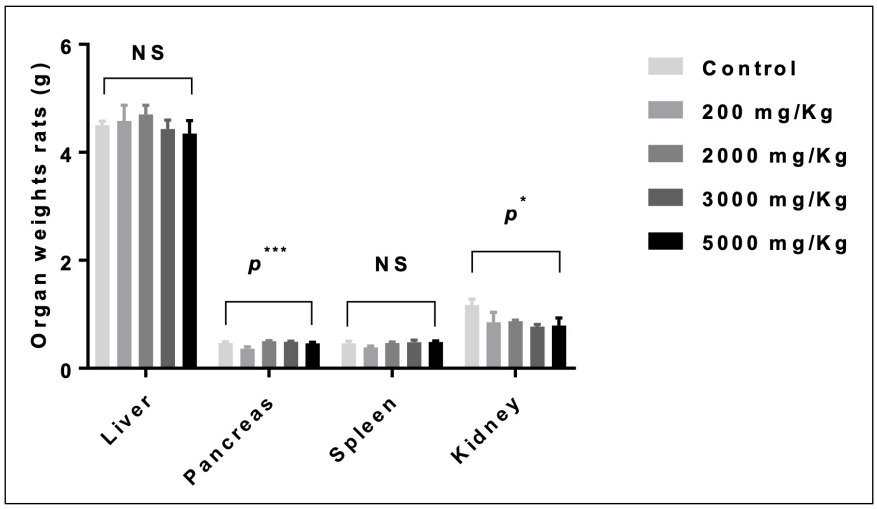

Fig. 2. Effect of methanol extract of Pomace Olive on organ weights rats (g) in the acute toxicity test.

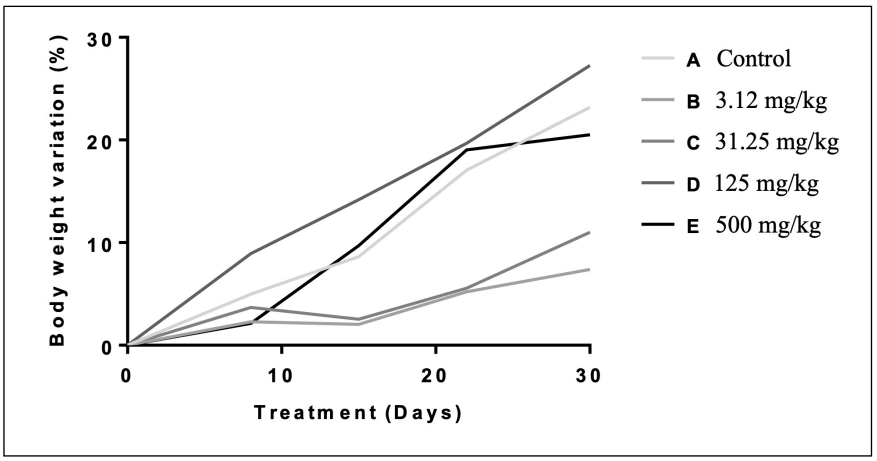

Fig. 3. Effect on the treatment with the methanol extract of olive pomace on the variation of body weight of rats in sub-acute oral toxicity test.

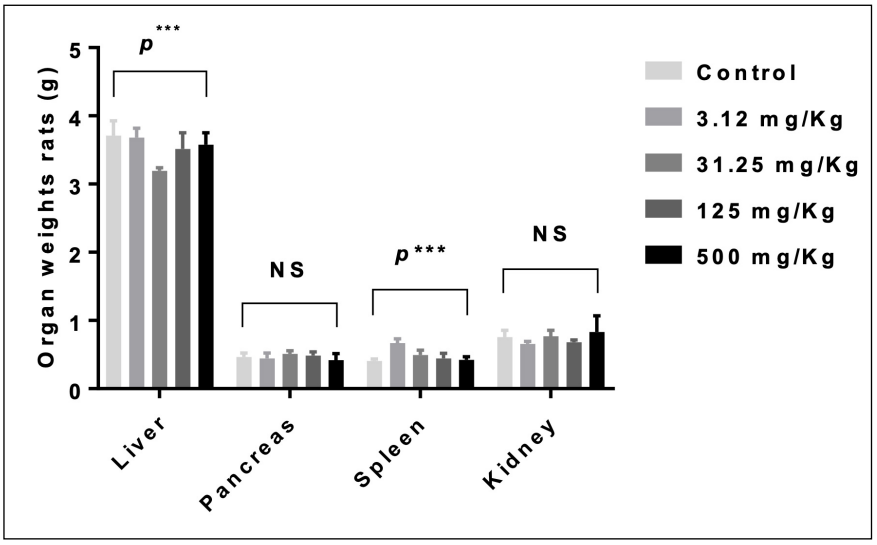

Fig. 4. Effect of methanol extract of olive pomace on organ weights rats (g %) in the sub-acute toxicity test.

TABLE 2. Effect of methanol extract of *Pomace Olive* on the biochemical parameters rats in the subacute toxicity study.

Biochemical parameters	Control (n= 4)	2000 mg/kg	500 mg/kg	125 mg/kg	31.25 mg/kg	3.12 mg/kg	p-value
Glucose (mg/dl)	144.977±0.76	125.200±1.00	132.449±1.154	144.14±3.99	144.08±1.79	143.70±2.21	**0.0001**
Total protein (g/dl)	4.013±0.444	3.717±0.197	3.213±1.075	3.923±0.231	4.7050±0.0450	3.513±0.960	0.1550
Trigliceride (mg/dl)	58.560±1.058	30.955±0.500	32.57±3.39	37.86±7.94	50.34±2.66	58.020±0.520	**0.0001**
Total cholesterol (mg/dl)	35.173±0.127	33.593±0.615	37.890±0.700	77.96±11.14	76.01±2.54	75.61±6.41	**0.0001**
AST (U/I)	75.640±0.753	179.350±1.15	159.51±9.09	70.62±9.69	54.420±0.516	72.25±4.55	**0.0001**
ALT (U/I)	34.737±0.491	141.873±1.62	97.00±7.09	49.900±0.600	37.76±1.74	27.63±2.07	0.3265
Urea(mg/dl)	31.48±2.33	140.963±0.77	121.73±6.27	63.4±21.9	48.303±0.415	31.94±2.02	**0.0001**
Creatinine (mg/l)	5.207±0.911	15.250±0.250	14.000±0.500	8.690±1.143	7.000±0.661	3.667±0.382	**0.0001**

For red blood cells, the results showed significant difference at dose 125 mg/kg and 31.25 mg/kg compared to control and the other treated groups; and on white blood cells had significant difference at doses 2000, 500 and 125 mg/kg ($p<0.05$).

On MCHC, analyses showed significant differences at doses 2000 mg/kg compared to control and all treatment doses.

On the eosinophils, the results showed significant differences at all treatment doses (Table 3).

Histological studies

A histopathological study carried to confirm biochemical findings and to identify any structural changes. Light microscopic examination of the vital organs including liver, kidney, and white adipose tissue of the rats in all the OP treated and control groups for acute oral toxicity (2000 mg/kg) (Figure 5) and sub-acute oral toxicity (Figure 6) did not reveal any gross pathological lesions.

The photomicrographs of the liver, kidney and adipose tissue of the control and OP treated groups showed with normal morphological architecture. Under microscopic examination of the liver of OP a treated animal showed with normal cellular architecture and binucleation and was without any distortions similar to the control groups. In white adipose tissue histological study, showed no significant adipogenesis was observed in all groups. Morphology of the adipocytes was maintained in a continuous polyhedral pattern with no significant variations in the adipocyte morphology and adipocyte intensity in compared to normal control sections rendering devoid of any alteration in adipose tissue.

The appearance of the glomerular architecture in real section showed normal similar to the control groups. The glomerul, distal and proximal tubules in the kidney appeared normal and no degeneration or necrosis in nephron cells in acute toxicity and sub-acute toxicity in dose 3.12, 31.25 and 125 mg/kg, furthermore at dose 500 mg/kg we notice acute inflammation with the predominant infiltrating cells in the neutrophil and lymphocytes into the medulla.

TABLE 3. Effect of methanol extract of *Pomace olive* on hematological parameters of rats in the acute toxicity (2000mg/kg) and sub-acute toxicity study.

	Control	2000 mg/kg	500 mg/kg	125 mg/kg	31.25 mg/kg	3.12 mg/kg	p-value
RBC (10^6/µl)	9.24±0.62	8.580±0.44	7.57±1.002	7.79±0.02	8.653±0.982	9.850±1.043	**0.0411**
Hematocrit(%)	41.93±3.19	40.85±1.65	28.08 ±3.79	30.5±0.99	32.60± 7.95	40.90±4.88	**0.021**
Hemoglobin(g/dl)	13.36±1.13	9.35±1.25	9.36±0.89	10.5±0.1	11.07±2.42	13.45±2.35	**0.0465**
VGM	45.3± 0.0.52		37.09±0.32	39.15±0.045	37.68±0.12	41.5±0.16	**0.035**
Platelets (10^3/µl)	775 ± 9.0	693.0± 5 8.0	638± 8.00	641.0±7.00	675.3±14.9	642±1.73	0.1057
MCH (pg)	17.71±0.35	18.3±0.8	19.20±1.01	19.4±0.2	19.46±0.50	18.600±1.32	0.1367
MCHC (g/dl)	34.56±0.67	37.90±0.60	24.97±2.54	24.45±0.45	27.63±2.15	35.40±0.79	**0.0128**
WBC (10^3/µL)	8.66±0.82	7.56±0.27	4.86±0.3	4.705±0.30	5.60±0.5	6.62± 0.69	**0.0115**
Neutrophils%	12.03±0.68	13.85±0.65	11.47±1.47	10.2±0.84	13.83± 1.02	14.07±2.27	0.5173
Lymphocytes%	85.33±1.48	80.15±0.495	81.8±3.3	80.85± 1.20	82.17± 1.57	85.70±2.60	0.3635
Monocytes%	2.86±1.04	2.25±0.35	2.60±0.91	2.20±0.14	2.50±0.21	2.57±0.650	0.5351
Basophils%	0.33±0.03	0.38±0.005	0.35±0.082	0.35±0.005	0.32±0.042	0.33±0.052	0.4874
Eosinophils%	1.33±0.14	1.4±0.1	2.04±1.76	3.95±0.15	3.11±0.10	3.23±1.305	**0.0071**

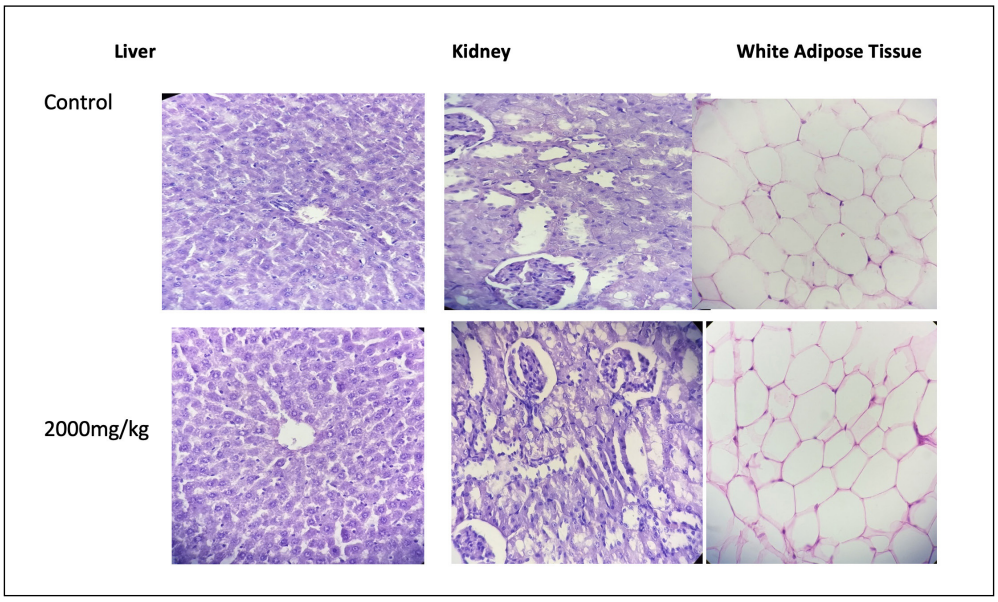

Fig. 5. Photomigrograph of vital organ (Liver, Kidney, White Adipose Tissue) in acute toxicity of Olive Pomace.

DISCUSSION

OP is considered to be a rich source of phenolic compounds[23-25], given the importance of phenolic bioactive compounds and their emergence as value-added materials with potential applications in the pharmaceutical, food and nutraceutical industries, the valorization of the certain agro-industrial by-product OP has drawn a significant amount of attention[24].

Thus, the present study is assumed to focus on the acute and sub-acute toxicity of methanol extract of OP. The qualitative phytochimecal analysis reveals the presence of polyphenols, flavonoids, tannins, saponins, quinones, anthraquinones, terpenoids and compounds reducing; these results are similar to those realized by Xie et al[4].

These phytocompounds acted as many pharmacological activities, which have been proved antiviral, antimicrobial, antioxidant, anti-inflammatory activities[26].

So, OP shows excellent results, and it is, therefore, necessary to perform the toxicity study of OP in order to evaluate its pharmacological safety *in vivo*.

The main objective of regulatory toxicology studies is to establish the potential hazards associated with the test item by identifying potential organ toxicity and are used to gather information on certain properties (e.g. pharmacokinetics) that, if inappropriate for humans, could prevent further development of the potential new medicine and, therefore, further animal use[27].When studying acute and sub-acute toxicity, the oral route administration is the most convenient and commonly used one, the absorption might be slow, but this method costs less and is painless to the animals. Since the crude extract is administered orally, the animals should be fasted before taking the dose because food and other chemicals in the digestive tracts may affect the reaction (s) of the compound[28].

In the present study, oral administrations of methanol extract of OP in the dose 2000 mg/kg for the acute toxicity do not produce any significant physical and behavioral changes and no death is recorded in the extract of OP. In the present study, extract dose of 3.12, 31.25, 125 and 500 mg/kg body weight of methanolic extract of OP, do not expose rats to a single dose of methanolic extract of OP (up to 2000 mg/kg), do not produce any mortality and alterations in body weight, adverse clinical signs, and during 14 days of the acute oral toxicity testing. So, it can be classified the methanol extract of OP as Category 5 with low acute toxicity hazard[29]. Since data obtained from acute toxicity is not sufficient to apply for clinical implication, sub-acute toxicity studies have significant importance in determining the safety profile of drugs or chemical moieties[30]. Although most of the plant-derived medicines show low acute toxicity, repeated dose toxicity is required to ascertain the possible toxic effects of a substance likely to arise from repeated administration over a limited period. Hence, the present study is undertaken to investigate the effects of methanol extract of OP after the oral administration in rats for 4-weeks. In the present investigation, oral administration of OP extract for 28 days does not show any mortality in rats which received produce any alterations in feed and water consumption and this reveals

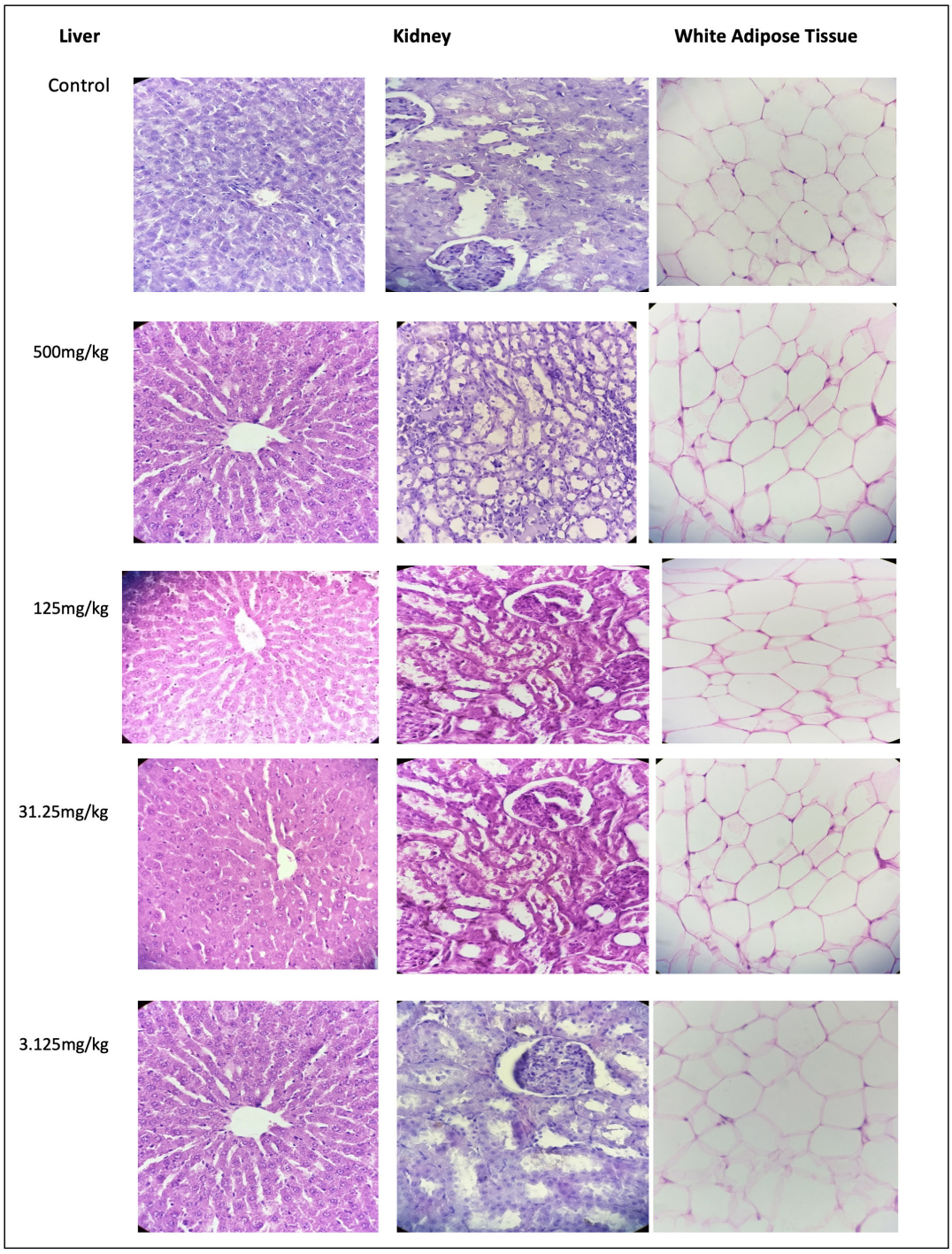

Fig. 6. Photomigrograph of vital organ (Liver, Kidney, White Adipose Tissue) in sub-acute toxicity of Olive Pomace.

that it does not adversely affect the basic metabolic processes of the experimental animals [28]. Body weight changes are an indicator of adverse side effects as the animals that survive cannot lose more than 10% of the initial body weight[31]; in our study we note evolution of body weight of rats (control and all groups treated) in both acute and sub-acute oral toxicity test, in like manner study performs by Etame-Loe et al[32] test acute and sub acute toxicity of the palm wine extract of seeds of Carica papaya Linn. Organ weight also is an important index of physiological and pathological status in animals. The relative organ weight is fundamental to diagnose whether the organ is exposed to the injury or not; the heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant [33]. During the analysis of the various organs, no significant alteration notes in the organ weights compared to the normal control organ weight.

The evaluation of the hematological parameters is very important in the determination of the anomalies induced by a plant extract [34], blood serves as the main medium of transport for many drugs and xenobiotic in the body and for that reason components of the blood exposed to substantial concentrations of toxic compounds. Damage and destruction of the blood cells are inimical to normal functioning of the body both in humans and animals [21]. In the present study treatment with PO does not produce any alteration in hematological parameters (platelets, MPV, MCH, neutrophils, monocytes, lymphocytes and basophils); however, the results show a significant difference in RBC, hematocrit, hemoglobin, WBC, MCHC and eosinophils at dose 500 and 125 mg/kg. The decrease in erythrocytes, leukocytes and MCHC indicate microcytic hypochromic anemia of rats. This condition might lead to anemia that impairs erythropoiesis caused by a direct effect of metals on hematopoietic centers (kidney/spleen), accelerated erythroclasia because of the altered membrane permeability and/or increased mechanical fragility, and defective Fe metabolism or failure of intestinal uptake of Fe because of mucosal lesions [35]. In herbal toxicity studies, increase in WBC may indicate the impact of plant extracts in inducing the 30 immune response of treated animals. On the other hand, significant decrease in the WBC of the blood indicates a decline in the production of leukocytes called leukopenia, means that the body is less able to fight off infections [36]. Clinical biochemistry data hold significant role in determining the toxicity induced by drugs [37]. The alterations in biochemical parameters in a toxicology study are described and analyzed using a reasonably standardized method and the fold-changes are usually described to indicate the extent of alterations, based on the degree of alterations of serum chemistry parameters, a biochemical interpretation is made, and additional tests recommended [38].

Liver and kidney are site of drug metabolism and elimination and most sensitive organs to be affected by chemical toxicity. Any alteration in liver or kidney functions result in the elevation of SGOT, SGPT, ALP, BUN, serum creatinine and electrolytes in blood [37]. In sub-acute toxicity, our study demonstrates a significant decrease in serum glucose at high dose 500 mg/kg compared to control. These results are in agreement with Cherrad et al [39], who found the hypoglycemic effect of PO in diabetic rats. In animal models studies, the hypoglycemic effect could be facilitated by the reduction of starch digestion and absorption. Moreover, hydroxytyrosol, oleuropein major phenolic compounds of PO have a hypoglycemic and antioxidant activity *in vitro* and in rats [40]. For transaminases which are good indicators of liver function and biomarkers to predict the possible toxicity, our results reveal rats that consumed high dose of PO (500 mg/kg) present a significant increase in serum AST and ALT compared to control rats. An increase of ALT suggests hepatocellular damage, while AST is less specific than ALT as an indicator of liver function. Altered permeability of the hepatocellular membrane caused by either injury or metabolic disturbance results in a release of soluble cytosolic enzymes. These enzymes generally escape out from the basal-lateral side of the hepatocytes facing the sinusoids causing their elevation in the blood [29,41].

Serum lipid profile shows a significant change compared to control; the data present a diminution of total cholesterol and triglycerides in rats administered with dose 500 mg/kg, these results are in accordance with Liu et al [6] who examined the hypolipidemic effect of OP extract on cholesterol regulation and LDL cholesterol oxidation; also, the results show a decrease of HDL in rats administered with high dose 500 mg/kg and increase of this parameter at dose 125 mg/kg. However, increase concentration of HDL is associated with decrease accumulation of atherosclerosis within the walls of arteries. This is because atherosclerosis results in sudden plaque ruptures, cardiovascular disease, stroke and other vascular diseases. Additionally, the decrease in HDL cholesterol observed in this study across the treatments could be similar with the study conducted on mice that showed HDL-lacking mice still have the ability to transport cholesterol to bile, suggesting that there are alternative mechanisms for cholesterol to be removed from the blood [42].

Proteins are the fundamental components of all living cells and include many substances such as enzymes, hormones and antibodies that are necessary for the proper functioning of an organism [27]. Our findings show no significant change in total protein of treated groups compared to control.

Measurement of plasma urea and creatinine has been used for many years as an indicator of kidney function [43]. In preclinical toxicity studies, renal changes are particularly liable to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites [44]. The current study presents a significant increase change in urea and creatinine compared to control. Urea is the metabolic product of protein catabolism and increases in serum urea might hamper the kidney function if it is not controlled accordingly [45]. Elevations of serum urea and creatinine show poor clearance by kidney, indicating damage to the renal tissue [46]. The evaluation of histopathological changes in organs remains a cornerstone in safety assessment of medicines [37]. The histological inspection on the treated and control groups shows that the methanol extract of OP does not cause any toxic effect in liv-

er, kidney and adipose tissue in acute toxicity and sub-acute toxicity in the doses 125, 31.25, 3.12 mg/kg. Because hepatic and renal function are crucial, it is essential to know the state of these two vital organs to evaluate the toxicity of any new compound [43] and adipose tissue is a potential site of toxicant accumulation [47]. However, we find that an acute interstitial inflammatory cell infiltrate is present in the renal medulla in 500 mg/kg. The toxicity observed in these studies could result from high dose of active organic constituents like saponins, tannins and flavonoids as shown by the result of phytochemistry [48].

CONCLUSIONS

The conclusion drawn from this study is that methanol OP extract in acute and sub-acute test causes any lethality and apparent toxicity. On the other hand, the results show alteration in some hematological parameters (glucose, triglycerides, total cholesterol, HDL, AST, urea and creatinine) besides renal, hepatic parameters and histological modification at dose 500 mg/kg. Additional research into chronic toxicity test and *in vitro* cytotoxicity activity of OP extract should also be achieved in the future.

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Conflict of interest:

The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

Ethics approval:

Experimental protocols adopted were based on World Health Organization Guidelines for care and use of laboratory animals. The experimental usage of the animals was approved by the Ethics Committee of the National Ethics Committee for Control and Supervision of Experiments on Animals.

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Contributions:

Badi Zouleykha: study design, data collection, data interpretation and wrote the manuscript. Guermouche Baya: collected data, searched literature and revised the manuscript critically. Haddam Nahida: established toxicological study design, data collection and helped draft the manuscript. Belayagoubi Nabila: performed the study design. Benzerjeb Hajira: performed histological study. Kechkouche Youcef: performed statistical study. Merzouk Hafida and Dali- Sahi Majda: participated in the study design, data interpretation, manuscript preparation. All authors declare that they contributed to this article and that they read and approved final version.

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